COVID-19: urgent reconsideration of lung edema as a preventable outcome
Inhibition of TRPV4 as a promising and feasible approach

Wolfgang M. Kuebler[1], Sven-Eric Jordt[2], Wolfgang B. Liedtke[2,3,4]

Institute of Physiology[1], Charité Medical University of Berlin, Germany
Duke University, Departments of Anesthesiology[2], Neurology[3] and Neurobiology[4], Durham NC, USA

Correspondence: wolfgang.kuebler@charite.de, sven.jordt@duke.edu, liedt001@duke.edu
lead-contact: liedt001@duke.edu

Summary:

Lethality of Covid-19 during the 2020 pandemic, currently in the exponentially-accelerating phase in most countries, is critically driven by disruption of the alveolo-capillary barrier of the lung, leading to lung edema as a direct consequence of SARS-CoV-2 infection. We argue for inhibition of the TRPV4 calcium-permeable ion channel as a strategy to address this issue, based on the rationale that TRPV4 inhibition is protective in various preclinical models of lung edema, and that TRPV4 hyperactivation potently damages the alveolo-capillary barrier, with lethal outcome. We believe that TRPV4 inhibition has a powerful prospect at protecting this vital barrier in Covid-19 patients, even to rescue a damaged barrier. A clinical trial using a selective TRPV4 inhibitor demonstrated a benign safety profile in healthy volunteers and in patients suffering from cardiogenic lung edema. We argue for expeditious clinical testing of this inhibitor in Covid-19 patients with respiratory malfunction and at risk for lung edema. We note that among the currently pursued therapeutic strategies against Covid-19, none is designed to directly protect the alveolo-capillary barrier. Successful protection of the alveolo-capillary barrier will not only reduce Covid-19 lethality but will pre-empt a catastrophic scenario in healthcare with insufficient capacity to provide ventilator-assisted respiration.

At this point (early April, 2020), true lethality of SARS-CoV-2 infections is unknown because the accurate number of infected remains undetermined (1). Any effective reduction in lethality of severe Covid-19 is urgently needed on a global scale. In particular, interventions to reduce the numbers of patients requiring assisted ventilation is critical since their numbers threaten to exceed the available ventilator capacity, a potentially catastrophic perspective (2, 3).
Here we propose the calcium-permeable TRPV4 ion channel as a currently underappreciated yet promising target for efforts to reduce respiratory complications, morbidity and mortality of severe Covid-19. Clinically feasible inhibition of TRPV4 should be seriously and urgently considered as a rapidly implementable new treatment for this purpose.

The case for TRPV4 is based on these 3 lines of evidence
1) Fortunately, the severity of the majority of Covid-19 cases is not amounting to a level of critically ill. However, once SARS-CoV-2 infection progresses to the stage of pneumonia, then alveolo-capillary barrier failure and ensuing formation of permeability-type lung edema become key pathogenic drivers toward critically-ill SARS. This clinical stage shows characteristic symptoms of the acute respiratory distress syndrome (ARDS) and can readily lead to lethal outcome. This clinical path of manifest SARS transitioning to critically-ill SARS via alveolo-capillary barrier failure is a shared hallmark of SARS-CoV-1, SARS-MERS and SARS-CoV-2 caused diseases (4-9).

2) TRPV4 channels are multimodally-activated calcium-permeable cation channels that have been identified as important regulators of alveolo-capillary barrier integrity, with expression in all relevant cell types of the alveolo-capillary unit, namely alveolar type I and type II cells as well as alveolar capillary endothelial cells (10-13). In addition, TRPV4 is expressed in and regulates the activation of innate immune cells such as alveolar macrophages and neutrophil granulocytes which contribute to alveolo-capillary barrier disruption via the release of proteases, cytokines, and reactive oxygen species (11, 14-21). The critical role of TRPV4 in alveolo-capillary barrier integrity was first documented in 2006 in a study demonstrating that selective TRPV4 activation results in rapid loss of alveolo-capillary barrier function and subsequent formation of alveolar edema (22), also see our **Figure**. Since then, the particular role of TRPV4 in alveolo-capillary barrier regulation has been corroborated and extended in a series of preclinical studies showing protective effects of TRPV4 inhibition in models of permeability-type lung edema following e.g. mechanical overventilation, acid aspiration, or chlorine inhalation (11, 13, 15, 17, 18, 23). It was also demonstrated that TRPV4 regulates alveolo-capillary barrier integrity in a human lung-on-a-chip model (24). In several preclinical studies including one in primates, selective TRPV4 inhibitors were shown to prevent or attenuate cardiogenic lung edema following acute left ventricular failure (25). On the other hand, a nano-molar potency TRPV4 selective activator caused lethality in several experimental animal species upon systemic injection by causing endothelial barrier failure with subsequent acute circulatory collapse and pulmonary edema (26). Recently, a gene-therapeutic approach was employed to suppress TRPV4 function via a co-expressed protein, the CD98 high-homology domain (27). Using AAV gene-therapy viral vectors for transduction of human lung cells, this approach proved effective to attenuate alveolo-capillary barrier failure in a lung-on-a-chip model. Finally, exosomes derived from human adipocytes have been found to protect mice against ventilator-induced lung injury via inhibition of TRPV4-mediated calcium influx (28). Taken together, there is ample evidence for a protective effect of TRPV4 inhibition on alveolo-capillary barrier function, and different pharmacological and genetic approaches have been developed for effective targeting of TRPV4.

3) Inhibition of TRPV4 in Covid-19 patients is clinically feasible with inhibitor compounds available for clinical testing and implementation now. One particular inhibitor, GSK2798745, has been

tested in Phase-I trials in human healthy volunteers, also in patients with cardiogenic lung edema and chronic cough, and was found to be safe in all cohorts (29-33).
Importantly, compared to classic ARDS which develops acutely and as such, does not allow for preventive measures, Covid-19 is characterized by a gradual onset of symptoms which progress in a slow-crescendo from flu-like symptoms to pneumonia and ultimately, respiratory failure (3). As such, the Covid-19 scenario is ideally suited for adjunctive therapies such as inhibition of TRPV4. A TRPV4 inhibitor can be introduced into patients early after the onset of respiratory symptoms before their progression to a SARS-like clinical picture with the aim to stabilize the alveolo-capillary barrier prior to its failure. Early protection of the alveolo-capillary barrier prior to overt alveolar flooding may prove to be particularly life-saving in regions where the needed medical infrastructure (number of ventilator beds, operated by competent intensive care teams) is insufficient vs number of patients in need with critically-ill SARS. As such, TRPV4 inhibition appears as an attractive and feasible strategy to alleviate the global burden of deaths from Covid-19 which otherwise could rapidly become highly challenging. With development of Covid-19 countermeasures focusing on antivirals, vaccines, protease-inhibitory and immunomodulatory drugs, treatment with a TRPV4 inhibitor has an encouraging outlook to achieve additive or even synergistic effects (34-39).

Commentary and caveats

We argue for rapid consideration of TRPV4 inhibitory therapy that can be implemented essentially NOW for effective protection of the alveolo-capillary barrier of Covid-19 patients.
Despite its clear promise, some potential restrictions and caveats should not go unmentioned: First, systemic inhibition of TRPV4 could prove problematic due to potential effects on hepatic function, a concern that is particularly relevant in the critically ill (40), yet a hepato-protective role of TRPV4 inhibition has also been postulated in acetaminophen toxicity (41). Notably, phase-I clinical trials using a selective TRPV4 inhibitor did not detect increased liver enzymes in healthy volunteers or patients with congestive heart failure (30). In regards to respiratory infections, recent studies report that TRPV4 inhibition may reduce bacterial clearance of *Pseudomonas aeruginosa* by macrophages (42). However, TRPV4 inhibition seems beneficial in lung infection with *Streptococcus pneumoniae*, the most frequent microbial cause of community-acquired pneumonia (43). In addition, an earlier study documenting the role of TRPV4 in alveolar barrier integrity in-vivo demonstrated that ventilator-induced lung injury depended on TRPV4 function in macrophages (17, 18).
While these potential caveats, rooted in preclinical studies with divergent results, need to be subjected to scrutiny in future studies, we believe that they are outweighed by the urgent need for alveolo-capillary barrier-stabilizing drugs in the present Covid-19 pandemic.
Rapid clinical trials will be daunting, but do-able as the example of currently ongoing clinical trials with remdesivir in Covid-19 illustrates (44).
Taken together, we reiterate the global and urgent priority to reduce Covid-19-associated lethality and potentially disastrous burden on health care systems due to the need for ventilator-assisted respiration. We identify TRPV4 as a worthy target to protect and rescue the integrity of the alveolo-capillary barrier as an achievable milestone to avoid extremely unfortunate outcomes. Addressing this task under normal circumstances would perhaps take years. We do not have this time now and need to adapt regulatory schedules to our unprecedented current situation.

References

1. Yang J, Zheng Y, Gou X, Pu K, Chen Z, Guo Q, et al. Prevalence of comorbidities in the novel Wuhan coronavirus (COVID-19) infection: a systematic review and meta-analysis. Int J Infect Dis. 2020.
2. Baud D, Qi X, Nielsen-Saines K, Musso D, Pomar L, Favre G. Real estimates of mortality following COVID-19 infection. Lancet Infect Dis. 2020.
3. Zhou F, Yu T, Du R, Fan G, Liu Y, Liu Z, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. Lancet. 2020.
4. Ketai L, Paul NS, Wong KT. Radiology of severe acute respiratory syndrome (SARS): the emerging pathologic-radiologic correlates of an emerging disease. J Thorac Imaging. 2006;21(4):276-83.
5. Hsiao CH, Wu MZ, Chen CL, Hsueh PR, Hsieh SW, Yang PC, et al. Evolution of pulmonary pathology in severe acute respiratory syndrome. J Formos Med Assoc. 2005;104(2):75-81.
6. Hwang DM, Chamberlain DW, Poutanen SM, Low DE, Asa SL, Butany J. Pulmonary pathology of severe acute respiratory syndrome in Toronto. Mod Pathol. 2005;18(1):1-10.
7. Chow KC, Hsiao CH, Lin TY, Chen CL, Chiou SH. Detection of severe acute respiratory syndrome-associated coronavirus in pneumocytes of the lung. Am J Clin Pathol. 2004;121(4):574-80.
8. Franks TJ, Chong PY, Chui P, Galvin JR, Lourens RM, Reid AH, et al. Lung pathology of severe acute respiratory syndrome (SARS): a study of 8 autopsy cases from Singapore. Hum Pathol. 2003;34(8):743-8.
9. Tian S, Hu W, Niu L, Liu H, Xu H, Xiao SY. Pulmonary Pathology of Early-Phase 2019 Novel Coronavirus (COVID-19) Pneumonia in Two Patients With Lung Cancer. J Thorac Oncol. 2020.
10. Yin J, Hoffmann J, Kaestle SM, Neye N, Wang L, Baeurle J, et al. Negative-Feedback Loop Attenuates Hydrostatic Lung Edema via a cGMP-Dependent Regulation of Transient Receptor Potential Vanilloid 4. Circ Res. 2008.
11. Yin J, Michalick L, Tang C, Tabuchi A, Goldenberg N, Dan Q, et al. Role of Transient Receptor Potential Vanilloid 4 in Neutrophil Activation and Acute Lung Injury. Am J Respir Cell Mol Biol. 2016;54(3):370-83.
12. Goldenberg NM, Ravindran K, Kuebler WM. TRPV4: physiological role and therapeutic potential in respiratory diseases. Naunyn Schmiedebergs Arch Pharmacol. 2015;388(4):421-36.
13. Morty RE, Kuebler WM. TRPV4: an exciting new target to promote alveolocapillary barrier function. Am J Physiol Lung Cell Mol Physiol. 2014;307(11):L817-21.
14. Zhao P, Lieu T, Barlow N, Sostegni S, Haerteis S, Korbmacher C, et al. Neutrophil Elastase Activates Protease-activated Receptor-2 (PAR2) and Transient Receptor Potential Vanilloid 4 (TRPV4) to Cause Inflammation and Pain. J Biol Chem. 2015;290(22):13875-87.
15. Balakrishna S, Song W, Achanta S, Doran SF, Liu B, Kaelberer MM, et al. TRPV4 inhibition counteracts edema and inflammation and improves pulmonary function and oxygen saturation in chemically induced acute lung injury. Am J Physiol Lung Cell Mol Physiol. 2014;307(2):L158-72.
16. Scheraga RG, Abraham S, Niese KA, Southern BD, Grove LM, Hite RD, et al. TRPV4 Mechanosensitive Ion Channel Regulates Lipopolysaccharide-Stimulated Macrophage Phagocytosis. J Immunol. 2016;196(1):428-36.
17. Hamanaka K, Jian MY, Townsley MI, King JA, Liedtke W, Weber DS, et al. TRPV4 channels augment macrophage activation and ventilator-induced lung injury. Am J Physiol Lung Cell Mol Physiol. 2010;299(3):L353-62.
18. Hamanaka K, Jian MY, Weber DS, Alvarez DF, Townsley MI, Al-Mehdi AB, et al. TRPV4 initiates the acute calcium-dependent permeability increase during ventilator-induced lung injury in isolated mouse lungs. Am J Physiol Lung Cell Mol Physiol. 2007;293(4):L923-32.
19. Pairet N, Mang S, Fois G, Keck M, Kuhnbach M, Gindele J, et al. TRPV4 inhibition attenuates stretch-induced inflammatory cellular responses and lung barrier dysfunction during mechanical ventilation. PLoS One. 2018;13(4):e0196055.
20. Dutta B, Arya RK, Goswami R, Alharbi MO, Sharma S, Rahaman SO. Role of macrophage TRPV4 in inflammation. Lab Invest. 2020;100(2):178-85.

21. Rayees S, Joshi JC, Tauseef M, Anwar M, Baweja S, Rochford I, et al. PAR2-Mediated cAMP Generation Suppresses TRPV4-Dependent Ca(2+) Signaling in Alveolar Macrophages to Resolve TLR4-Induced Inflammation. Cell Rep. 2019;27(3):793-805 e4.
22. Alvarez DF, King JA, Weber D, Addison E, Liedtke W, Townsley MI. Transient receptor potential vanilloid 4-mediated disruption of the alveolar septal barrier: a novel mechanism of acute lung injury. Circ Res. 2006;99(9):988-95.
23. Michalick L, Erfinanda L, Weichelt U, van der Giet M, Liedtke W, Kuebler WM. Transient Receptor Potential Vanilloid 4 and Serum Glucocorticoid-regulated Kinase 1 Are Critical Mediators of Lung Injury in Overventilated Mice In Vivo. Anesthesiology. 2017;126(2):300-11.
24. Huh D, Leslie DC, Matthews BD, Fraser JP, Jurek S, Hamilton GA, et al. A human disease model of drug toxicity-induced pulmonary edema in a lung-on-a-chip microdevice. Sci Transl Med. 2012;4(159):159ra47.
25. Thorneloe KS, Cheung M, Bao W, Alsaid H, Lenhard S, Jian MY, et al. An Orally Active TRPV4 Channel Blocker Prevents and Resolves Pulmonary Edema Induced by Heart Failure. Sci Transl Med. 2012;4(159):159ra48.
26. Willette RN, Bao W, Nerurkar S, Yue TL, Doe CP, Stankus G, et al. Systemic activation of the transient receptor potential vanilloid subtype 4 channel causes endothelial failure and circulatory collapse: Part 2. J Pharmacol Exp Ther. 2008;326(2):443-52.
27. Li J, Wen AM, Potla R, Benshirim E, Seebarran A, Benz MA, et al. AAV-mediated gene therapy targeting TRPV4 mechanotransduction for inhibition of pulmonary vascular leakage. APL Bioeng. 2019;3(4):046103.
28. Yu Q, Wang D, Wen X, Tang X, Qi D, He J, et al. Adipose-derived Exosomes Protect the Pulmonary Endothelial Barrier in Ventilator-induced Lung Injury by Inhibiting the TRPV4/Ca2+ Signaling Pathway. Am J Physiol Lung Cell Mol Physiol. 2020.
29. Brooks CA, Barton LS, Behm DJ, Eidam HS, Fox RM, Hammond M, et al. Discovery of GSK2798745: A Clinical Candidate for Inhibition of Transient Receptor Potential Vanilloid 4 (TRPV4). ACS Med Chem Lett. 2019;10(8):1228-33.
30. Goyal N, Skrdla P, Schroyer R, Kumar S, Fernando D, Oughton A, et al. Clinical Pharmacokinetics, Safety, and Tolerability of a Novel, First-in-Class TRPV4 Ion Channel Inhibitor, GSK2798745, in Healthy and Heart Failure Subjects. Am J Cardiovasc Drugs. 2019;19(3):335-42.
31. ClinicalTrials.gov UNLoM 2018; NCT02119260
32. Stewart GM, Johnson BD, Sprecher DL, Reddy YNV, Obokata M, Goldsmith S, et al. Targeting pulmonary capillary permeability to reduce lung congestion in heart failure: a randomized, controlled pilot trial. Eur J Heart Fail. 2020.
33. ClinicalTrials.gov UNLoM 2019;NCT03372603
34. Chan KW, Wong VT, Tang SCW. COVID-19: An Update on the Epidemiological, Clinical, Preventive and Therapeutic Evidence and Guidelines of Integrative Chinese-Western Medicine for the Management of 2019 Novel Coronavirus Disease. Am J Chin Med. 2020:1-26.
35. de Wit E, Feldmann F, Cronin J, Jordan R, Okumura A, Thomas T, et al. Prophylactic and therapeutic remdesivir (GS-5734) treatment in the rhesus macaque model of MERS-CoV infection. Proc Natl Acad Sci U S A. 2020.
36. Morse JS, Lalonde T, Xu S, Liu WR. Learning from the Past: Possible Urgent Prevention and Treatment Options for Severe Acute Respiratory Infections Caused by 2019-nCoV. Chembiochem. 2020;21(5):730-8.
37. Wang M, Cao R, Zhang L, Yang X, Liu J, Xu M, et al. Remdesivir and chloroquine effectively inhibit the recently emerged novel coronavirus (2019-nCoV) in vitro. Cell Res. 2020;30(3):269-71.
38. Lu H. Drug treatment options for the 2019-new coronavirus (2019-nCoV). Biosci Trends. 2020;14(1):69-71.
39. Hoffmann M, Kleine-Weber H, Schroeder S, Kruger N, Herrler T, Erichsen S, et al. SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor. Cell. 2020.

40. Seth RK, Das S, Dattaroy D, Chandrashekaran V, Alhasson F, Michelotti G, et al. TRPV4 activation of endothelial nitric oxide synthase resists nonalcoholic fatty liver disease by blocking CYP2E1-mediated redox toxicity. Free Radic Biol Med. 2017;102:260-73.
41. Echtermeyer F, Eberhardt M, Risser L, Herzog C, Gueler F, Khalil M, et al. Acetaminophen-induced liver injury is mediated by the ion channel TRPV4. FASEB J. 2019;33(9):10257-68.
42. Scheraga RG, Abraham S, Grove LM, Southern BD, Crish JF, Perelas A, et al. TRPV4 Protects the Lung from Bacterial Pneumonia via MAPK Molecular Pathway Switching. J Immunol. 2020;204(5):1310-21.
43. Michalick L, Kuebler WM**.** TRPV4-A Missing Link Between Mechanosensation and Immunity. Front Immunol. 2020;11:413.
44. Wikipedia 2020;https://en.wikipedia.org/wiki/Remdesivir

Figure:

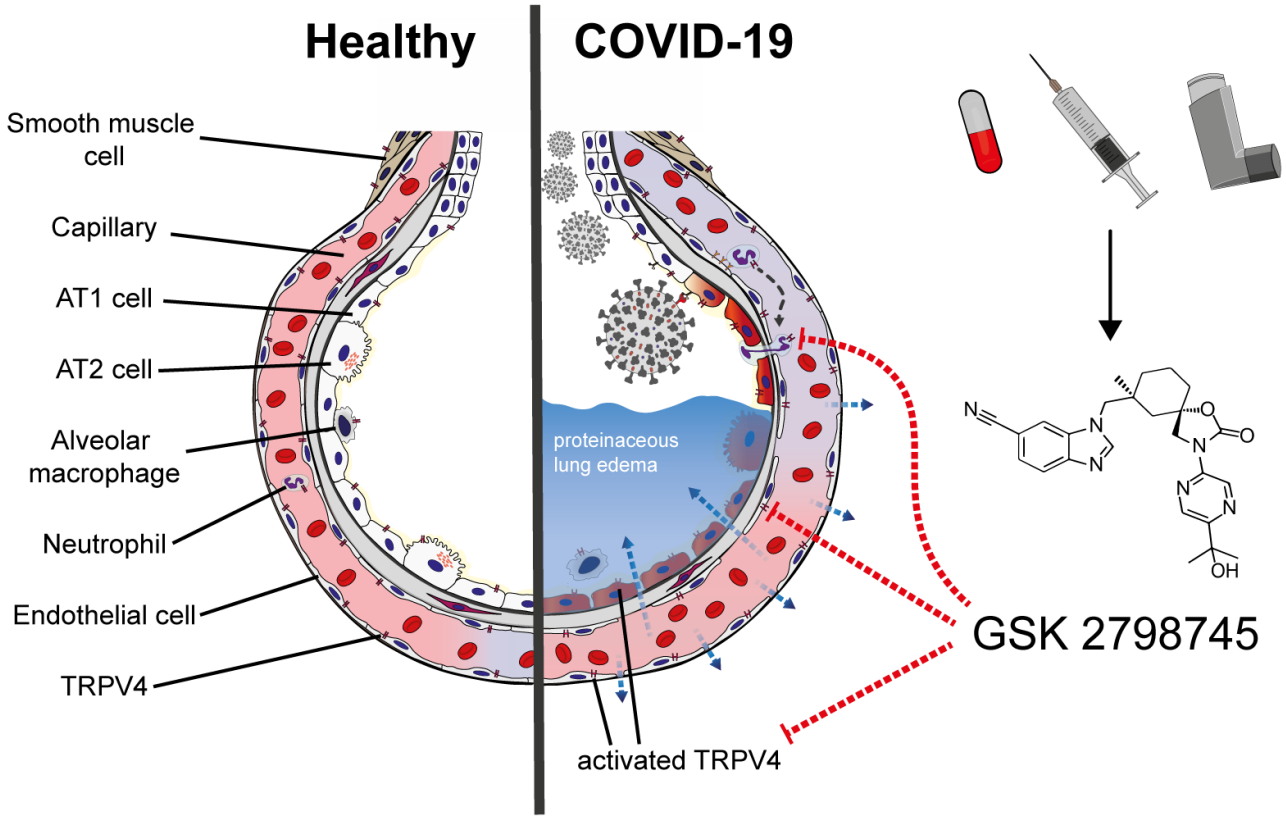

Figure: Alveolo-capillary barrier, TRPV4 expression and proposed treatment with GSK2798745 in Covid-19.
Note that TRPV4 expression and function might differ under lung infection with SARS-CoV-2. As we argue, we believe that the benefits outweigh the risks, from making possibly transformative therapeutic inroads against Covid-19 – at the individual patient and especially at the population health level – by protecting the alveolo-capillary barrier by inhibiting TRPV4.

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Conflict of Interest: Wolfgang Liedtke co-founded TRPblue, a biotechnology start-up company that is aiming to commercialize TRPV4/TRPA1 dual-inhibitory compounds for treatment of chemotherapy-associated nerve pain and chronic allergic skin inflammation. Of note, none of TRPblue's compounds would be suitable for the advocated approach because they await testing in humans and are intended for topical application to skin.

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There is no additional conflict of interest.